Altered Muscle Metabolism in Rats After Thermal Injury

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Burn injury is associated with an elevation in total body oxygen consumption, increased hepatic alanine uptake and conversion to glucose, and a negative nitrogen balance. The primary source of the alanine used for gluconeogenesis by the liver and of the nitrogen lost as urea is believed to be from skeletal muscle. Selected muscle regulatory enzymes and pyruvate and oleate oxidation rates were assayed for maximal activity during the postburn period. Male Sprague-Dawley rats that received 50% total body surface scald burns on the dorsum and abdomen were examined for citrate synthase (CS), phosphofructokinase (PFK), and glutamate-pyruvate transaminase (GPT) activity in uninjured muscle at 3, 7, 13, and 20 days postburn, and the ability of muscle to oxidize pyruvate and oleate was measured at 3 and 13 days after injury. CS, PFK, and GPT activities increased significantly ($p < 0.05$) by 13–20 days after injury in the soleus and diaphragm. The epitrochlearis showed no change in CS, but PFK and GPT were elevated within this time frame. The gastrocnemius muscle showed an elevated oleate oxidation rate at 13 days after injury, but no change at 3 days postburn. Pyruvate oxidation rates were unaltered.

The results of this study indicate that during the postburn period several metabolic alterations occur in muscle. These adaptations include: (1) elevated CS activity which may be associated with increased oxidative capactiy, (2) increased PFK activity which implies that more substrate is being shuttled through the glycolytic pathway, (3) increased GPT activity which may reflect increased pyruvate conversion to alanine, and (4) increased oleate oxidation rates which demonstrate that muscle is utilizing more fatty acid substrates during the postburn period.

BURN INJURY causes dramatic, acute, and chronic physiological alterations. Burn patients exhibit a hypermetabolic period which reaches its zenith on the 9th to 12th postburn day,[1,2] and the degree of this hypermetabolic response is proportional to the extent and severity of the wound.[1,3] Tissues from both injured and non-injured extremities are thought to contribute to the elevation in whole-body oxygen consumption during the hypermetabolic period.[1] The elevated oxygen consumption present after burn injury implies that more energy is being utilized by the burn patient and that more substrate must be oxidized to provide this energy. Therefore, it is important to determine if this prolonged increase in metabolic rate causes adaptations in the uninjured tissue's ability to utilize and/or provide energy and substrates.

The postburn period has been characterized by several metabolic alterations. The burn patient exhibits an elevated hepatic glucose production and increased hepatic alanine uptake after injury.[4] Peripheral glucose utilization has been shown to be unaffected until 6–16 days after injury, at which time the rate of glucose disappearance is elevated.[5] During this period the glucose-alanine cycle proceeds at an accelerated rate[6] and the nitrogen from alanine and other amino acids is lost as urea at an increased rate, resulting in a negative nitrogen balance.[4,6] Skeletal muscle is believed to be the primary source of these amino acids that are used as gluconeogenic precursors by the liver during the postburn period.[4,6]

Skeletal muscle is known to adapt to changes in its metabolic state. Exercise,[7,8] selected hormonal treatment,[9] and cold-stress[10] are all effectors of muscle metabolic adaptations. Muscle has the ability to adapt to these stimuli by selectively increasing the amount of mitochondrial protein, while other protein structures, such as the myofibrils, do not always exhibit a net increase in muscle fiber protein content.[7,8] This adaptation is a selective process involving an altered expression of protein synthesis. The unburned limb of an injured patient has an elevated oxygen consumption during the postburn hypermetabolic period,[1] and this response may be due to increased skeletal muscle oxygen utilization resulting from increased muscle mitochondrial protein and oxidative capactiy. Exposure to some stimuli after burn injury may cause the muscle to adapt to its new environment and utilize more oxygen. The purpose of this study is to determine whether muscle exhibits measurable metabolic adaptations during the postburn period. Selected regulatory enzymes involved in glycolysis, amino acid metabolism, and the citric acid cycle were examined.

MATERIALS AND METHODS

Animal Care and Treatment

Male Sprague-Dawley rats were used in this investigation and were maintained on a diet of Purina laboratory chow and water provided ad libitum and exposed to a 12:12 hr light-dark cycle. The animals were divided into two groups: a control group and a burned

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In conducting the research described in this report, the investigators adhered to the "Guide for the Care and Use of Laboratory Animals," as promulgated by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council.

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group. The animals were burned using the procedure by Herndon et al.[11] Briefly, this procedure consists of anesthetizing the rat (50 mg pentobarbital per kg), shaving the area to be burned, placing the animal in a body mold which exposes a known percentage of the total body surface (TBS), and scalding the exposed area in water to produce the desired wound depth. In this experiment, the rats (180–200 g) received a 50% TBS burn (30% on the dorsum and 20% on the abdomen). In order to produce a full-thickness wound and minimize damage to underlying tissues, the dorsum was scalded for 9 sec and the abdomen for 3 sec in 98°C water. Saline (20 ml) was given i.p. prior to scalding the abdomen to provide protection to the viscera and to aid in the resuscitation of the animal. Animals from each group were sacrificed at 3, 7, 13, and 20 days postinjury. Controls were sacrificed with burned animals on these selected postburn days, and all experiments were performed at the same time of day.

Tissue Sampling and Processing

Selected muscles representing a broad spectrum of fiber types were removed from anesthetized (50 mg/kg, pentobarbital) rats on the specified postburn day. The soleus, epitrochlearis, and diaphragm were chosen for the enzyme studies. The soleus is classified as an intermediate fiber type muscle (intermediate oxidative capacity, low glycolytic capacity), the diaphragm is a "red" muscle (high oxidative capacity, moderate glycolytic capacity), and the epitrochlearis is a "white" muscle (low oxidative capacity, high glycolytic capacity). The gastrocnemius, a mixed fiber type muscle, was chosen for substrate utilization studies in order to obtain enough tissue to measure pyruvate and oleate oxidation rates from the same animals. The muscles for the enzyme studies were dissected free of connective tissue, minced on ice, and weighed prior to dilution for homogenization. A 5% (w/v) homogenate was prepared from each muscle with a medium consisting of 175 mM KCl, 10 mM glutathione, and 2.0 mM EDTA (pH 7.0). Each sample remained chilled during homogenization with an Ultra-Turrax homogenizer (Tekmar Ind., Cincinnati, Oh.).

A portion of the whole homogenate was centrifuged at 1,000 *g* for 10 min. The supernatant and remaining whole homogenate were stored at −80°C until analysis.

Enzyme Assays

All assays were performed in 1 ml cuvettes of 1 cm light path at 30°C. Reaction rates were measured at zero-order kinetics, and the rates were proportional to the protein concentration of the samples.

Citrate synthase, CS (citrate: oxaloacetate-lyase, EC 4.1.3.7), activity was assayed as described by Srere[12] with the use of 5,5′-dithiobis-(2-nitrobenzoic acid), DTNB. Final reagent concentrations used in this assay included 60 mM Tris buffer (pH 8.0), 300 μM acetyl-CoA, 100 μM DTNB, 500 μM oxaloacetate, 3.0 mM K_2HPO_4, and diluted homogenate equivalent to 0.1 mg wet weight of tissue.

Phosphofructokinase, PFK (ATP: D-fructose-6-phosphate 1-phosphotransferase, EC 2.7.1.11), activity was measured in the 1,000 g supernatant using the assay procedure described by Bergmeyer et al.[13] The final reagent concentrations in the reaction mixture were: 70 mM Tris buffer (pH 8.5), 1.4 mM $MgSO_4$, 4.5 mM KCl, 0.71 mM phosphoenolpyruvate, 0.64 mM fructose-1,6-diphosphate, 1.8 mM fructose-6-phosphate, 1.1 mM ATP, 0.4 mM NADH, 4.2 U/ml pyruvate kinase, 9.6 U/ml lactate dehydrogenase, and enough sample to provide 1.0 mg wet weight of tissue.

Glutamate-pyruvate transaminase, GPT (L-alanine: 2-oxoglutarate aminotransferase, EC 2.6.1.2), activity was measured using the spectrophotometric assay described by Bergmeyer and Bernt.[14] Optimum conditions included the following reagents in the assay mixture: 80 mM potassium phosphate buffer (pH 7.4), 18 mM α-ketoglutarate, 600 mM L-alanine, 0.18 mM NADH, 1.2 U/ml lactate dehydrogenase, and enough homogenate to provide 1.0 mg wet weight of tissue.

Sample protein content was determined using the biuret method described by Gornall et al.[15] Bovine serum albumin was used as the standard for determining protein concentrations.

Substrate Oxidation Assays

The capacity of whole homogenates of the gastrocnemius muscle to oxidize pyruvate-1-^{14}C to $^{14}CO_2$ was measured using the assay procedure described by Baldwin et al. Incubations were performed at 30°C in a shaking incubator. $^{14}CO_2$ was trapped with triethanolamine after CO_2 was liberated from the reaction suspension with 1.0 ml of 15% perchloric acid. The trapped $^{14}CO_2$ was counted in Econofluor (New England Nuclear, Boston, Mass.) and 10% methanol.

Oleate-1-^{14}C oxidation rates by whole gastrocnemius homogenates were determined utilizing the assay procedure described by Molé et al.[16] $^{14}CO_2$ was released, trapped, and counted as described above for the pyruvate-^{14}C oxidation assay.

Analysis of Results

The analysis for statistically significant alterations in enzyme activity over the postburn period was performed using a one-way analysis of variance for each muscle for each enzyme. The difference in activities between different fiber type muscles is well documented and was not of primary interest in this study.

RESULTS

Body Weight Response

Previous work from this laboratory determined that uninjured control male Sprague-Dawley rats gain 6–7 g/day over the 180–350 g body weight range.[11] The control animals in this study gained weight at 6.6 ± 0.5 g/day, but the burned group gained weight at 3.9 ± 0.4 g/day over the 20 day postburn period ($p < 0.01$). The muscle weights of the burned rats were less than controls. At 13 days postinjury the burned group had soleus weights of 96 ± 6 mg and the control values were 123 ± 5 mg ($\bar{X}$ ± SEM). The body weights of the rats at 13 days after injury were 276 ± 12 g for the burned group and 345 ± 7 g for the control group. The burned group had lower body weights and muscle weights, but the muscle:body weight ratios were similar between groups—.35 and .36 for burned and control groups, respectively.

Enzyme Activities

Citrate synthase activity was measured in each muscle (Table 1). An increase in citrate synthase activity was observed in the soleus (13%) and diaphragm (17%) of burned rats at 13 days postinjury and the levels of citrate synthase remained elevated at 20 days postburn. The epitrochlearis muscle showed no significant change in

Table 1. Citrate Synthase Activity in Uninjured Rat Muscle After Burn Injury

	Soleus	Diaphragm	Epitrochlearis
	(nmoles/min/mg protein)		
Controls (6)	110.3 ± 7.3	187.3 ± 12.2	82.8 ± 3.8
3 days postburn (10)	113.4 ± 7.7	190.3 ± 6.1	74.9 ± 6.3
7 days postburn (5)	118.0 ± 3.4	192.0 ± 8.0	77.5 ± 5.3
13 days postburn (10)	123.6 ± 3.9	219.0 ± 8.7*	85.3 ± 4.1
20 days postburn (5)	135.2 ± 9.8*	201.8 ± 16.0	79.7 ± 6.2

Values are means ± SEM; number of () is *n* per group.
*$p < 0.05$ versus controls.

Table 2. Phosphofructokinase Activity in Uninjured Rat Muscle After Burn Injury

	Soleus	Diaphragm	Epitrochlearis
	(nmoles/min/mg protein)		
Controls (6)	34.3 ± 1.8	80.3 ± 4.6	147.2 ± 5.0
3 days postburn (10)	35.8 ± 1.3	80.1 ± 7.5	130.6 ± 8.1
7 days postburn (5)	30.1 ± 1.7	70.2 ± 2.4	128.4 ± 7.3
13 days postburn (10)	39.2 ± 2.8	94.8 ± 5.8*	163.5 ± 6.0
20 days postburn (5)	43.8 ± 3.0*	100.2 ± 5.6*	174.2 ± 13.3*

Values are means ± SEM; number in () is *n* per group.
*$p < 0.05$ versus controls.

citrate synthase activity over the postburn period. The protein content per gram wet weight of muscle in the injured animals was not significantly different from control values at any postburn time period, indicating that no significant edema or change in protein concentration occurred within these muscles after injury.

Phosphofructokinase (PFK) activity was assayed in each muscle at selected postburn time periods (Table 2). PFK activity in the soleus and diaphragm was significantly higher (28% and 25% respectively) by the 20th postburn day. The epitrochlearis showed a 17% increase in PFK by the 20th postburn day.

Glutamate-pyruvate transaminase (GPT) activity was assayed in each muscle during the postinjury period (Table 3). The GPT activity in the soleus and diaphragm maximized by the 13th–20th postburn day, with the GPT activity increasing 52% in the soleus and 39% in the diaphragm. By the 13th day after injury GPT activity in the epitrochlearis increased 50% above control levels.

Substrate Utilization

The ability of whole gastrocnemius homogenates to oxidize pyruvate-^{14}C and oleate-^{14}C was examined at 3 and 13 days postinjury (Table 4). No significant differences in oxidation rates for pyruvate or oleate were observed 3 days after injury, but by 13 days after injury the ability of the gastrocnemius to oxidize oleate was increased significantly (29%) above control levels. Pyruvate oxidation rates at 13 days postburn were not statistically different.

A portion of the gastrocnemius homogenates was saved for determination of CS activity. At 13 days postinjury the control group CS activity was 11.7 ± 0.8 and the burned group was 13.9 ± 1.09 SEM (μmoles/min/g). This is a significant 16% increase in CS activity in the burned group of animals. This increase in CS corresponds with similar increases observed in the other muscles studied.

DISCUSSION

Thermal injury causes several metabolic alterations in uninjured skeletal muscle. In this study the burned animals had a significantly slower growth rate and

Table 3. Glutamate-Pyruvate Transaminase Activity in Uninjured Rat Muscle After Burn Injury

	Soleus	Diaphragm	Epitrochlearis
	(nmoles/min/mg protein)		
Controls (6)	20.6 ± 1.4	36.3 ± 1.2	24.8 ± 0.9
3 days postburn (10)	24.6 ± 1.7	35.1 ± 2.4	22.3 ± 1.9
7 days postburn (5)	28.1 ± 2.3	38.1 ± 3.1	30.1 ± 1.6
13 days postburn (10)	30.3 ± 1.7*	50.2 ± 3.2*	36.6 ± 2.4*
20 days postburn (5)	31.4 ± 2.6*	50.4 ± 3.4*	31.4 ± 2.7*

Values are means ± SEM; number in () is *n* per group.
*$p < 0.05$ versus controls.

Table 4. Pyruvate-^{14}C and Oleate-^{14}C Oxidation Rates by Whole Gastrocnemius Homogenates

	Pyruvate-^{14}C (μmoles/hr/g)	Oleate-^{14}C (nmoles/min/g)
Controls (5)	98.2 ± 6.7	2.19 ± 0.10
3 days postburn (5)	84.2 ± 3.5	1.85 ± 0.29
13 days postburn (5)	87.2 ± 4.0	2.82 ± 0.34*

Values are means ± SEM; number per group in ().
*$p < 0.05$ versus controls.

smaller muscles, in comparison to uninjured control rats. These results confirm previous observations that showed injured animals or patients with unlimited access to food do not maintain their preinjury weight.[11,17] These results have been interpreted[18] to mean that the burned animal does not have the ability to utilize enough substrate to produce energy and/or precursors for wound healing and anabolic metabolism simultaneously.

The measurement of maximal enzyme activity in this study provides information regarding a change in enzyme protein content in muscle. A change in maximal enzyme activity is generally caused by an alteration in the rate of enzyme protein synthesis and/or degradation, assuming no specific inhibition or activation of enzyme activity occurs. This technique is commonly employed in many types of investigation.[16,19-21] The maximal activity data is not a direct measure of substrate flux through metabolic pathways; however, several investigators have shown that increased pathway activity over an extended period of time often results in changes in the maximal activity of enzymes within that pathway.[22-25] The primary purpose of this investigation was to determine whether the infliction of thermal injury causes an adaptive response in the maximal activities of selected enzymes.

Selected rat muscles were examined for changes in maximal enzyme activity over the postburn period. The enzymes chosen for study were citrate synthase, glutamate-pyruvate transaminase, and phosphofructokinase. Citrate synthase is a citric acid cycle enzyme located in the micochondrial matrix and is often used as a marker for mitochondrial content in muscle.[7,8] Glutamate-pyruvate transaminase is a cytoplasmic and mitochondrial enzyme involved in the transfer of amino groups from free intracellular amino acids to pyruvate to form alanine. Phosphofructokinase is a cytoplasmic glycolytic regulatory enzyme involved in the oxidation of glucose and/or glycogen through the glycolytic pathway.

In this investigation an elevation in the specific activity of citrate synthase was observed in the soleus and diaphragm muscles by 13 days after injury. This observation indicates that these muscles are responsive to some stimulus during the postburn period which causes a change in citrate synthase synthesis and/or

degradation. If citrate synthase reflects the response of other citric acid cycle enzymes and represents a general enzyme adaptation to injury, such would be consistent with an increase in mitochondrial content in muscle. Elevated mitochondrial content increases the oxidative capacity of the muscle and, therefore, the ability of the muscle to use oxygen to produce ATP. The elevation in citrate synthase and oxidative capacity of these muscles may be the mechanism responsible for the elevated oxygen consumption measured across an uninjured limb in burn patients.[1] The data from pyruvate and oleate oxidation experiments (Table 4) support the contention that elevated citrate synthase reflects higher muscle oxidative capacity. The time frame for this adaptation to occur in burned rats is similar to the time required for the development of the hypermetabolic zenith in burn patients. Both hypermetabolism in patients and the increase in citrate synthase in rat muscle are evident by 13 days after injury.

Both GPT and PFK follow a time course of adaptation similar to CS in the soleus and diaphragm. GPT activity increases to significantly higher levels by 13 days postinjury, indicating that the maximal capacity for producing alanine from pyruvate increases significantly in these muscles by 13 days after injury. These results provide evidence that alanine is lost from muscle at a faster rate during this period. Burn patients exhibit elevated alanine in the blood, as well as higher hepatic alanine uptake and glucose production,[6] and other investigators have hypothesized that this alanine comes from skeletal muscle.[4,6] These results provide some evidence that muscle does produce higher amounts of alanine as reflected by increases in GPT activity after burn injury.

The carbon source employed for the production of the alanine which is lost from muscle is unclear. Several investigators believe that the carbon is derived from carbohydrate precursors which are degraded to pyruvate and then transaminated to form alanine,[26,27] while others believe that the alanine is derived from endogenous muscle protein breakdown.[28]

The elevated levels of muscle PFK activity observed in this study after burn injury also provide further evidence that the capacity for glucose and/or glycogen utilization increases after injury. Although glycolysis generates relatively little energy, the pyruvate formed from glycolysis can be oxidized in the citric acid cycle, transaminated to form alanine, or reduced to form lactate. Since GPT and CS activity are elevated in muscle after injury, it is possible that the majority of the pyruvate is used for transamination to form alanine and fatty acids are oxidized preferentially as the main energy source. This idea is supported by the data for pyruvate and oleate oxidation (Table 4), whereby oleate is oxidized at an elevated rate and pyruvate oxidation is unaltered at 13 days postinjury.

Interestingly, the epitrochlearis muscle did not show an increase in CS during the postburn period. However, GPT and PFK were elevated by 13–20 days after injury. These results indicate that the oxidative capacity of the epitrochlearis was unaltered, but the capacity for glycolysis and transamination was elevated. The epitrochlearis is classified as a "white" fiber type muscle.[29] White muscles have a very low oxidative capacity and derive most of their energy from glycolysis. Generally, white muscles are not recruited to aid in movement unless the movement is vigorous,[7,8] whereas "red" and "intermediate" mucles are employed constantly to maintain posture and perform everyday movements such as walking and eating. Furthermore, the white muscles are poorly vascularized and the red and intermediate muscles are well vascularized.[8]

In the burned animal, the contractile activity of the diaphragm, soleus, and epitrochlearis muscles varies from constant to very slight, respectively. The muscles that are recruited more frequently (diaphragm and soleus) are also the muscles that show an increase in citrate synthase activity after injury. Unlike the burn patient, an injured rat must feed itself and appears to maintain its posture after resuscitation, thereby using the soleus muscle more than would be expected in the burn patient. This maintenance of neuromuscular tone may be a prerequisite for maintenance of normal rates of protein synthesis in skeletal muscle. The lack of muscle contractile activity due to limb immobilization has been shown to cause a significant decrease in skeletal muscle mitochondrial protein and oxidative capacity.[30] These results may indicate that a certain degree of neuromuscular tone is required for adaptive increases in oxidative capacity in muscle, but the degree of this increase does not seem to be proportional to the level of muscle contractile activity since the postburn increase in citrate synthase activity in the soleus and diaphragm is approximately the same in both muscles. The epitrochlearis (a white muscle) showed no increase in citrate synthase activity after injury, and its contractile activity is minimal during slow postural movements.

The gastrocnemius muscle (a mixed fiber type muscle) showed an enhanced capacity to oxidize oleate at 13 days postinjury. The increase in the oxidation rate of oleate corresponds with an increase in CS activity measured in the soleus and diaphragm muscles. These results indicate that the gastrocnemius muscle has adapted during the postburn period by increasing its ability to oxidize substrate and produce energy. Pyruvate oxidation was not elevated, indicating that the

gastrocnemius muscle was utilizing the fatty acid oleate preferentially. The respiratory quotient (RQ) in burned and injured patients has been shown to decrease during the postinjury period in the absence of dietary intervention,[1] and these results suggest that the preferential utilization of fatty acids by skeletal muscle contributes to this decrease in RQ.

The results of this study indicate that metabolic adaptations occur in uninjured muscles in response to thermal injury. These adaptations maximize at approximately the same time as the whole body hypermetabolic response reaches its zenith. Red and intermediate muscles show adaptive increases in oxidative capacity, whereas white muscle does not demonstrate an increase in citrate synthase activity within the same time frame as the red and intermediate fiber type muscles. By 13 days after injury, muscle oxidizes oleate at a faster rate than muscle from control animals and oleate appears to be oxidized in preference to pyruvate. The fact that uninjured muscles show adaptations after burn injury provides evidence that some pervasive signal is causing changes in muscle metabolism.

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